REMARKS

Reconsideration is respectfully requested.

The Examiner has withdrawn claims 14-16, 19-26, 29-33, 39, and 44-46, as being directed to non-elected subject matter. On entry of this amendment, claims 1-10, 12, 17, 18, 27, 28, 34-37, 40, 43, and 47 are amended. Claims 11 and 13 are reiterated. Claim 48 has been added. Claims 41 and 42 were cancelled in the Preliminary Amendment filed September 20, 2000. Claims 1-13, 17, 18, 27, 28, 34-38, 40, 43, and 47-48 are pending and under examination.

Claims 1, 17, 18, and 27, and claims depending therefrom, have been amended to clarify the subject matter of the claims. The claims have been amended to specify that deoxyribonucleotide molecules are introduced to the animal cell, and that the methods and synthetic genes are directed to post-transcriptionally repressing, delaying, or otherwise reducing the expression of a target gene. Support for deoxyribonucleotides may be found at, for example, in the specification at page 22, line 11. Support for post-transcriptionally repressing, delaying, or otherwise reducing expression may be found, for example, at page 1, lines 7-8 if the Specification. Claim 18 has been amended to move the limitation "promoters" to a dependent claim.

Claims 35 has been amended to correct an incorrect claim dependency, and to include the limitation of a "promoter."

Claim 36 has been amended to more clearly specify the claimed subject matter.

Claims 43 and 47 have been amended to exclude humans.

Claim 48 has been added to specify that the promoter sequence is the CMV promoter sequence.

With respect to all amendments and cancelled claims, Applicants have not dedicated or abandoned any unclaimed subject matter and moreover have not acquiesced to any rejections and/or objections made by the Patent Office. Applicants reserve the right to pursue prosecution of any presently excluded claim embodiments in future continuation and/or divisional applications.

In all the amendments, no NEW MATTER has been entered.

Restriction/Election

Applicants acknowledge the finality of the Examiner's restriction requirement.

Claim Rejections - 35 USC § 101

The Examiner rejects claims 43 and 47 under 35 U.S.C. 101 alleging that the invention is directed to non-statutory subject matter.

Claims 43 and 47

Claims 43 and 47, as amended, are directed to "an animal or human cell, animal or human tissue, animal or human organ."

The Examiner's Rejection

The Examiner argues that the claims, as they are directed to a whole organism comprising a synthetic gene, encompass a genetically modified human, which is not patentable subject matter.

Applicant's Response

Applicants have amended claims 43 and 47 to include an "animal cell, tissue, organ or non-human animal." The claims therefore no longer refers to a genetically modified human.

In view of the claim amendments, this ground for rejection is now moot. Applicants respectfully request that it be withdrawn.

Rejections under 35 USC § 112, first paragraph

I. Rejection of claims 1-13, 17, 18, 43 and 47 under 35 U.S.C. § 112

The Examiner rejects claims 1-13, 17, 18, 43 and 47 under 35 U.S.C. 112, first paragraph. Applicants respectfully traverse, since the specification not only fully enables the claims, but there is no basis in fact for the Examiner's rejection.

A. The Claims

Amended claims 1 and 18, and, through their dependency, claims 2-13, 17, 43, and 47, are directed to "post-transcriptionally repressing, delaying or otherwise reducing the expression of a target gene in an animal cell, tissue or organ." Further, the method is directed to "introducing ...one or more deoxyribonucleotide molecules." (Emphasis added).

Claims 43 and 47 are directed to "an animal or human cell, animal or human tissue, animal or human organ, or whole animal comprising" a "synthetic gene" (claim 43) or a "genetic construct" (claim 47). Both claims depend from claim 27, which is directed to "a synthetic gene comprising deoxyribonucleotide molecules ...wherein the synthetic gene is capable of post-

transcriptionally repressing, delaying, or otherwise reducing expression of the target gene." (Emphasis added).

B. The Legal Standard

Under 35 U.S.C. §112 ¶ 1, a patent specification containing a teaching of how to make and use the invention must be taken as enabling unless the PTO provides sufficient reason to doubt the accuracy of the disclosure. *In re Marzocchi*, 439 F.2d 220, 223-224, 169 U.S.P.Q. 367, 369-370 (C.C.P.A. 1971).

The claimed invention as disclosed in the specification cannot be questioned on the unsupported skepticism of the Office. *Ex parte Linn*, 123 U.S.P.Q. 262 (PTO Bd. Pt. App. Int. 1959); *Ex parte Rosenwald*, 123 U.S.P.Q. 261 (PTO Bd. Pt. App. Int. 1959) (emphasis added). The number and variety of examples is irrelevant in the disclosure is "enabling" and set forth the "best mode contemplated." Even in an unpredictable art, Section 112 does not require disclosure of a test of every species encompassed by the claims. *In re Angstadt*, 190 U.S.P.Q. 214, 218 (C.C.P.A. 1976).

An invention is enabled even though the disclosure may require some routine experimentation to practice the invention. *Hybritech v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 U.S.P.Q. 81, 94 (Fed. Cir. 1986). The fact that the required experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *ML T v. A.B. Fortia*, 774 F.2d 1104, 227 U.S.P.Q. 428 (Fed. Cir. 1985). A considerable amount of experimentation is permitted if it is merely routine or the specification provides a reasonable amount of guidance and direction to the experimentation. *In re Wands*, 858 F.2d 731, 8 U.S.P.Q.2d 1400 (Fed. Cir. 1988); In re *Jackson*, 217 U.S.P.Q. 804, 807 (PTO Pt. Bd. App. Int. 1982) (emphasis added). Finally, the Office has the burden of showing that the disclosure entails undue experimentation. *In re Angstadt*, 537 F.2d 498, 190 U.S.P.Q. 214 (CCPA 1976) (emphasis added).

There are many factors to be considered when determining whether there is sufficient evidence to support a determination that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is "undue." These factors include, but are not limited to: 1) the breadth of the claims, 2) the nature of the invention, 3) the state of the prior art, 4) the level of one of ordinary skill, 5) the level of predictability in the art, 6) the amount of direction

provided by the inventor, 7) the existence of examples, and 8) the quantity of experimentation needed to make or use the invention based on the content of the disclosure. *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

C. Meeting the Legal Standard

The Examiner has failed to meet the requisite prima facie burden of showing that the rejected claims lack enablement. Moreover, the Examiner fails to provide any factual support for the contention that the claims are not enabled or would require undue experimentation.

The claims are directed to methods of using deoxyribonucleic acid constructs in what has become known, since the filing date of the present patent application, as "RNA interference," or RNAi. (See attached Dykxhoorn, D. M., Novina, C. D. & Sharp, P. A., Killing the messenger: short RNAs that silence gene expression. Nature Reviews Molecular Cell Biology, June (2003)). In RNAi, expression of a target gene is post-transcriptionally repressed, delayed, or reduced by a double stranded RNA (dsRNA), where the sequence of each strand is substantially identical to the sequence of at least a region of a target gene. The interfering dsRNA can be two separate strands of RNA which then hybridize, or a single stranded RNA sequence including two self-complementary sequences. The dsRNA may be administered directly. Alternatively, the RNA maybe formed in vivo from a DNA construct. In the present patent application, Applicants' claimed method is limited to the latter approach, i.e. repressing, delaying, or reducing the expression of a target gene introducing a DNA molecule encoding dsRNA to the animal cell, tissue, or organ, instead of directly transfecting dsRNA. Specifically, Applicants' claimed RNAi method is directed to repressing, delaying, or reducing the expression of a target gene by introducing the a deoxyribonucleic acid sequence that encodes at least two copies of a structural gene sequence, each copy of which is substantially identical to the nucleotide sequence of a target gene or region thereof, and wherein one copy of the structural gene sequence is in the sense orientation and the other copy is in the antisense orientation. The claim limitations are thus directed to methods and synthetic genes for inducing RNAi. Instead of following traditional methods of directly transfecting cells with dsRNA, the present methods are directed to introducing DNA encoding the interfering dsRNA.

RNAi differs fundamentally from other methods of inhibiting RNA expression, both in terms of observed effect, and in terms of mechanism. As discussed in the attached Dykxhoorn et al. reference, dsRNA is processed by an enzyme called Dicer, and binds a nuclease complex to

form an RNA-induced silencing complex (RISC). The RISC targets the target gene transcripts by base-pairing and cleaving the mRNA. In contrast to other distinct knockdown methods such as antisense RNA, specific gene silencing by RNAi is achieved quickly and easily.

The claims in the present application are not directed to traditional antisense methods. Although RNAi is often compared to antisense RNA, it is an entirely different process with an entirely different mechanism of action. Antisense RNA is a single stranded RNA molecule that hybridizes to the mRNA transcript of a target gene. The single stranded RNA inhibits translation of the mRNA by preventing translation of the mRNA transcript at the ribosome. Antisense methods carry numerous limitations, including single stranded nuclease digestion of single stranded antisense RNAs, poor uptake by cells, low expression or limited stability of complementary RNAs which result in nonspecific targeting or low efficiency of target inhibition, and poor uptake by cells, tissue, or organs, limiting the ability of the antisense RNA to reach its target site in the cell. See, for example, Dietz et al., U.S. Patent No. 5,814,500 (column 1, line 58 through column 2, line 4), cited by the Examiner. RNAi, by contrast, is double stranded and not vulnerable to single strand RNase digestion. Further, RNAi functions by binding dsRNA to a nuclease complex to form the RISC, which then targets mRNA transcripts.

There is was no factual support in the state of the art at the time of the invention to indicate that a person of ordinary skill in the art would not be able to make and use the presently claimed methods. Some investigators speculated that gene suppression in animal cells or animals would result in an immune response in mammalian cells, but this speculation not only lacked factual support, but was limited to direct transfection of double stranded RNA (dsRNA), as are the pending claims. First, Andrew Fire stated in a review article that in introducing dsRNA directly to cells, "the simple protocols used for invertebrate and plant systems [were] unlikely to be effective." (A. Fire, TIG September 1999, Vol. 15 No. 9: 358, 363), cited by the Examiner. Fire admitted that his doubts were speculative at best and lacked factual support, stating that "the hope that RNA-triggered silencing would exist in vertebrates... is not ruled out by any current data." (A. Fire, TIG September 1999, Vol. 15 No. 9: 358, 363; emphasis added). Fire also stated that there is "the possibility that some components of RNA-triggered silencing machinery could be conserved from lower organisms." (A. Fire, TIG September 1999, Vol. 15 No. 9: 358, 363). Fire's says nothing about an immune response in higher organisms when DNA encoding dsRNA is transfected *in vivo*.

Second, Fire's statements were limited to introducing RNA to cells and organisms, not introducing DNA, as claimed in the present application. Fire's speculation has been directly controverted in the scientific literature for DNA expressed dsRNA *in vivo*. For example, in Brummelkamp et al. (296 Science 550 (2002)) (included in the IDS attached hereto) the authors conducted experiments mirroring those of the present patent application. They successfully observed silencing by introducing a DNA molecule comprising two copies of a structural gene sequence corresponding to a target gene, as claimed herein. First, Brummelkamp et al. introduced a nucleic acid sequence encoding a synthetic gene comprising a nucleic acid sequence that is substantially identical to the nucleotide sequence of a target gene (see Brummelkamp, page 551, Figure 1A). Northern blot analysis revealed that the construct expressed both sense and antisense RNA (see Brummelkamp, page 551, column 2). The genetic constructs were capable of silencing p53. No interferon effect was observed.

In fact, numerous studies in the art have demonstrated the effectiveness of the methods claimed in the present invention. The effectiveness of the methods herein has been demonstrated repeatedly. In 2002, RNAi was the molecule of the year in Science magazine. Moreover, numerous companies make DNA molecules encoding dsRNA for repressing, delaying, or otherwise reducing expression of a target gene.

The Applicants provide more than sufficient direction to one of skill in the art to achieve the claimed methods without undue experimentation. In particular, the Specification provides direction necessary for one of skill in the art to accomplish each claimed method step.

Applicants provide detailed directions for introducing "one or more dispersed or foreign deoxyribonucleotide molecules." For example, the Specification discloses at page 38, line 22, that "standard methods described supra may be used to introduce synthetic genes and genetic constructs into the cell." The specification also discloses that conventional methods of administering synthetic genes may be used, including "liposome-mediated transfection or transformation, transformation of cells with attenuated virus particles or bacterial cells, cell mating, transformation or transfection procedures known to those skilled in the art or described by Ausubel et al. (1992)."

Applicants also provide detailed directions for making a "deoxyribonucleotide molecule" or "synthetic gene," "including at least two copies of a structural gene sequence that is substantially identical to a target gene or region thereof." Applicants provide numerous figures

containing two copies of a structural gene sequence. For example, Figures 23, 25, 39, 52, 53, 54, and 62-67 all disclose vectors with two copies of a structural gene sequence. In addition, Figure 24 depicts a vector with opposing promoter sequences.

Applicants further provide detailed directions to one of ordinary skill in the art to make a "deoxyribonucleotide sequence operably linked to a promoter sequence," as required by claim 18. The specification defines and discloses examples of promoter sequences from page 30, line 30 through page 33, line 5. The specification further describes operably linking the promoter to gene sequences molecules from page 33, line 7 through page 34, line 12. In addition, Figures 23, 25, 39, 52, 53, 54, and 62-67 all depict vectors having tandem repeats operably linked to a promoter. Figure 24, for example, depicts a vector having opposing promoters.

Since each claimed method step, and the constructs used therein, are provided in detail in the present Specification, one of skill in the art would not be required to conduct undue experimentation in order to practice the claimed invention.

Applicants' specification also provides examples demonstrating development of synthetic genes, as well as methods of inactivation utilizing the claimed methods. In particular, example 9 describes a method of post-transcriptionally repressing the α-1,3-galatosyltranferase (*GalT*) gene in animal cells. Silencing of *GalT* was selected since "it adds a variety of α-1,3-galatosyl groups to a range of proteins on the surface of cells," and is therefore easily assayed. Transformation methods disclosed by Applicants hardly require undue experimentation.

In view of the breadth of the claims, the nature of RNAi as opposed to other RNA based methods of gene suppression, direction provided by Applicants, and demonstrated success in the art, one of skill in the art would not be required to conduct any undue experimentation, to succeed in practicing the claimed methods.

C. Argument Advanced by the Examiner

The arguments advanced by the Examiner fail to make a prima facie case for a lack of enablement. Specifically, the Examiner makes several arguments that do not apply to a method of introducing "introducing one or more deoxyribonucleotide molecules," and fails to provide factual support for a lack of enablement. In addition, the Examiner makes some additional arguments that mischaracterize the claims and impermissibly read limitations from both the specification and the scientific literature into the claims.

First, the Examiner argues that "based on the teachings of the specification, the claims relate to a method of making a cell, tissue, or organ having novel traits and specifically having reduced expression of α-1,3-galactosyl transferase." The Examiner assets, in the absence of any support, that the "only patentably useful purpose of which is xenotransplantation." The Examiner further asserts that "although the instant specification does not state a purpose for suppression of α-1,3-galactosyl transferase expression, it is know in the art that the enzyme is required for expression of the major antigen responsible for hyperacute, antibody mediated rejection of xenografts."

The Examiner impermissibly reads limitations from scientific literature into the claims. The claims are not directed to a method of xenotransplantation. The claims are directed to methods of post-transcriptionally repressing, delaying, or reducing the expression of a target gene in an animal cell, tissue, or organ, and are supported by the specification. Repressing, delaying, or reducing the expression of a target gene encoding α-1,3-galactosyl transferase is a dependent claim of the method. Applicants describe α-1,3-galactosyl transferase in the Specification as a non-limiting example of reducing expression. While it is conceivable that the claimed methods could be used in xenotransplantation, the claims themselves are not limited to a method of xenotransplantation.

Second, the Examiner argues that the art at the time of filing only provides one example of gene suppression in an animal *in vivo*. The Examiner cites Fire (1999) Trends. Genet. 15:358-363, in which the author states that the art of record at the time of filing provides only one example of gene suppression in an animal (i.e. C. *elegans*) *in vivo* (see Fire (1999) *Trends. Genet.* 15:358-363).

As pointed out above and reiterated here, Fire's statements were directed to direct transfection of double stranded RNA, not introducing DNA molecules including at least two copies of structural gene sequence, as claimed herein. Fire's assertions thus do not apply to the claims of the present invention. Further, Fire's skepticism was entirely speculative and made in the absence of data. Specifically, Fire et al. asserts that "the simple protocols used for invertebrate and plant systems are unlikely to be effective," without providing any factual support. The Fire article goes on to point out that "nonetheless, a recent report of co-suppression in mammalian cells, and the implication of RNA triggers with a potentially double-stranded character in a number of natural genetic interference processes (X-inactivation and imprinting)

suggest the possibility that some components of RNA triggered silencing machinery could be conserved from lower organisms."

Actual data (as opposed to unsupported speculation) demonstrates that the interferon effect anticipated by Fire et al. does not occur when DNA is introduced directly to the cell, tissue, or organ. Specifically, Brummelkamp et al. (296 Science 550 (2002)), attached herein, discloses post-transcriptional repression, delay, or reduction of the expression of a target gene by introducing DNA molecules, without the interferon effect described by Fire et al. The data of Brummelkamp et al. thus repudiates Fire's unsupported skepticism.

Third, the Examiner asserts that additional basis for unpredictability is found in teachings from the antisense art. Specifically, the Examiner sites Dietz (U.S. Patent No. 5,814,500), which teaches that many studies with antisense show that gene expression is suppressed by 80%-90% of the normal level, but that such reduction is not typically sufficient to reduce the biological effect, i.e., 10%-20% expression is sufficient to maintain the biological function sought to be suppressed.

Dietz et al. is entirely non-analogous art, and fails to provide any factual basis to indicate that the present claims are not enabled. The claimed methods differ fundamentally from antisense methods. In antisense methods, a single stranded nucleic acid hybridizes to a target sequence. In Dietz, "unmodified" or "intact" stem loop structures that do not have the nucleotide sequence of the target gene, flank the single stranded antisense nucleic acid. The single stranded portion of the antisense nucleic acid is identical to a portion of the target gene so that it can readily hybridize to the target sequence. If the RNA sequence corresponding to the target gene is double stranded, the antisense RNA will fail to hybridize to its RNA target.

The claimed methods, by contrast, are directed to repressing, delaying, or reducing expression of a target gene by introducing a DNA molecule including at least two copies of a structural gene sequence, one of which is in the sense orientation, and the other of which is in the antisense orientation. The DNA encodes dsRNA that represses, delays, or reduces expression of the gene in what has become known as RNA interference, or RNAi. RNAi proceed via a completely different biochemical pathway than antisense methods. Specifically, RNAi proceeds via a biochemical pathway in which RNA processing enzymes target double stranded RNA products to mRNA transcripts of the target genes. Unlike antisense methods, RNAi requires the production of double stranded RNA. Further, the present compositions utilized in the presently

claimed methods would not function effectively in traditional antisense methods, since the dsRNA molecules do not hybridize to mRNA transcripts in the absence of the RISC protein complex. In the present invention, the dsRNA transcription product of the DNA molecule introduced to the cell represses, delays, or otherwise reduces expression of the target gene by an entirely different mechanism than antisense RNA.

The Examiner also argues that Good et al. demonstrate that the operability of the claimed method *in vivo*, in any animal, depends on a number of factors beyond those stated above. Specifically, the Examiner argues that Good *et al.* (1997) *Gene Ther.* 4:45-54 teaches that the effective intracellular expression of small RNA therapeutics requires that the RNA be efficiently transcribed, stabilized against rapid degradation, folded correctly, and directed to the part of the cell where it can be most effective (Abstract).

Like Dietz, Good et al. provide no factual basis supporting a prima facie case for a lack of enablement. Good et al. is non-analogous art, since the methods taught by Good et al. do not apply to the pending claims. As discussed in detail above, the pending claims are directed to introducing DNA encoding two copies of a structural gene, the transcription product of which represses, delays, or reduces the expression of a target gene post-transcriptionally, in a process that has become known as RNAi since the filing of the present patent application. Good et al. only teach limits of antisense RNA, catalytic RNA (ribozymes), and high-affinity RNA ligands, termed 'aptamers' or 'decoys.'" (See Good et al., page 45, column 1). Good et al. make no reference to RNAi, which proceeds by a fundamentally different mechanism than any of the RNA methods cited by Good et al.

Moreover, many of the limitations addressed by Good et al. are not observed with the claimed methods. First, regarding the stability of RNA expressed by the deoxyribonucleic acid or synthetic genes of the instant claims, the RNA expressed by the DNA forms a double stranded structure, protecting it from the RNase degradation that plagues antisense techniques. Second, when the DNA molecule or synthetic gene is transcribed, secondary structure of the RNA is minimal, since dsRNA generally comprises two complementary RNA molecules. Thus, the secondary structure problems observed for ribozymes do not apply to RNAi. Third, the claimed methods do not require delivery of the DNA or expressed RNA to a specific, subcellular location. Thus, concerns articulated by Good et al. regarding targeting RNA to a specific location do not arise in the claims of the present invention.

Finally, the Examiner argues that "although the relative level of skill in the art is high, given the very high level of unpredictability in the art and the absence of any teaching in the disclosure to indicate that the instant method has addressed sources of unpredictability, the skilled artisan would not be able to make or use the instant claimed products and methods without undue experimentation." This statement is simply without any factual basis or support. First, the Examiner points to no factual support for "a very high level of unpredictability in the art." Instead, the Examiner only sites non-analogous art pertaining to antisense methods, ribozymes, and organelle-targeting technology. Second, the Examiner points to no reference directed to repressing, delaying, or otherwise reducing the expression of a target gene by introducing a DNA encoding dsRNA to a animal cell, tissue, or organ. Third, as discussed above, the claims are not unpredictable or difficult to follow, since instructions on how to follow every limitation of the claims are detailed in the Specification.

II. Rejection of Claims 27, 28, 36-38, 43 and 47 under 35 U.S.C. § 112

The Examiner rejects claims 27, 28, 36-38, 43 and 47 under 35 U.S.C. 112, first paragraph.

Claims 27, 28, 36-38, 43, and 47

Claim 27 is directed to "a synthetic gene comprising: a dispersed or foreign deoxyribonucleotide molecule includes at least two copies of a structural gene sequence." Claims 28, 36-38, 43, and 47 further limit claims 27.

The Examiner's Rejection

The Examiner alleges that the specification does not enable any and all synthetic genes or genetic constructs comprising a foreign nucleic acid molecule having at least two copies of a structural gene sequence that is substantially identical to a target gene placed under the control of a promoter.

The Examiner asserts that the claims are "directed to synthetic genes or genetic constructs having structural limitations but no functional limitations." The specification discloses that such constructs can be used to produce novel traits in a particular cell, tissue or organ. The Examiner further contends that the art, as exemplified by Selker (1999) *Cell* 97:157-160, teaches that suppression of target gene expression is not predictably tied to the structure. Selker teaches, "[s]ingle-copy sequences can experience PTGS even in haploid cells; thus, while commonly repeat associated, PTGS is not repeat induced ... Not all sequences appear capable of

triggering PTGS ... Not every transformant bearing PTGS-susceptible sequence shows silencing, even when multiple copies of the DNA are present" (first column on page 158). Selker fails to provide any factual support for this assertion.

Based on Selker's unsupported assertion, the Examiner contends that the claims encompass synthetic genes and genetic constructs that would not function. The Examiner further alleges that since there is no guidance in the specification or prior art how one should use synthetic genes or constructs which do not function, it would require undue experimentation to practice the invention.

Applicant's Response

The Examiner bases this rejection entirely on a single, unsupported disclosure by Selker et al. Selker et al. do not address post-transcriptionally repressing, delaying, or reducing the expression of a target gene by introducing a DNA molecule including at least two copies of a structural gene sequence to a cell, tissue, or organ, and provide only unsupported, generalized statements in reference to RNAi that fail to rise to the level of requiring undue experimentation.

First, the Examiner provides the single, factually baseless statement by Selker et al. Selker et al. provides no factual support, whether data or otherwise, to evidence this statement. Second, the generalized statements of Selker are ambiguous at best, and do not establish that undue experimentation would be required by one of ordinary skill in the art. Selker states that "not all sequences appear capable of triggering PTGS." This statement does not indicate that very few sequences are capable of triggering PTGS, nor does this statement mean that a few select sequences are capable of triggering PTGS. Selker fails to provide any factual basis to support this blanket statement.

Moreover, as described in the response to the rejection of (above), Applicants have thoroughly described the claimed methods, and in addition have provided a working example directed specifically to α-1,3-galactosyl transferase. References such as Brummelkamp et al. have demonstrated that the limitations cited by Fire et al. do not apply to methods of repressing, delaying, or reducing expression of a target gene by introducing DNA to cells, tissue, or organs. Non-analogous art directed to antisense, ribozymes, and other methods do not apply to the instant claims. In addition, each method step is thoroughly discussed in Applicants specification.

Since the statements of Selker et al. are ambiguous, the Examiner has provided no factual evidence that one of skill in the art would require undue experimentation to make or use the

invention, and Applicant has provided extensive disclosure in how practice the invention, Examiner has failed to make the requisite prima facie burden of showing a lack of enablement.

Applicants respectfully request that this ground for rejection be withdrawn.

III. Rejection of Claims 34, 35 and 40 under 35 U.S.C. § 112

The Examiner rejects claims 34, 35 and 40 under 35 U.S.C. 112, first paragraph, as not properly enabled.

Claims 34, 35, and 40

Claim 34 is directed to "the synthetic gene according to claim 27 wherein the target gene is porcine α-1,3-galactosyltransferase gene."

Claim 35 is directed to "the synthetic gene according to claim 34, wherein promoter sequence is the CMV promoter sequence."

Claim 40 is directed to "the genetic construct according to claim 38 comprising plasmid pCMV.Galtx2 or pCMV.Galtx4."

The Examiner's Rejection

Although the art teaches that the products might be useful for preparing organs and tissues for xenotransplantation, the Examiner maintains that a skilled artisan would not be able to use the constructs for that purpose without first engaging in undue experimentation. The Examiner further argues that the "for the reasons described above, the skilled artisan would not be able to use the constructs for that purpose without first engaging in undue experimentation."

Applicants' Response

The Examiner impermissibly reads limitations from the scientific literature into the claims. The claims are not directed to a method of xenotransplantation. The claims are directed to methods of repressing, delaying, or reducing the expression of a target gene in an animal cell, tissue, or organ, and are supported by the specification. Silencing genes encoding α-1,3-galactosyl transferase is a dependent claim of the method. Applicants describe α-1,3-galactosyl transferase in the Specification as a non-limiting example of gene silencing, not xenotransplantation. While it is conceivable that the claimed methods could be used in xenotransplantation, the claims themselves are not limited to a method of xenotransplantation.

Moreover, as described in the response to the rejection of (above), Applicants have thoroughly described the claimed methods, and in addition have provided a working example

directed specifically to α-1,3-galactosyl transferase. References such as Brummelkamp et al. have demonstrated that the limitations cited by Fire et al. do not apply to methods of introducing DNA to cells, tissue, or organs. Non-analogous art directed to antisense, ribozymes, and other methods do not apply to the instant claims. Moreover, each method step is thoroughly discussed in Applicants specification. Selker et al. also do not address introducing a DNA molecule to cells, and provide only ambiguous, unsupported, generalized statements.

Since the Examiner has impermissibly read limitations from the scientific literature into the claims, and the claims are otherwise fully described and enabled by the Specification, the Examiner has failed to make the requisite prima facie burden of showing a lack of enablement.

Applicants respectfully request that this ground for rejection be withdrawn.

Rejection under 35 USC § 112, second paragraph

The Examiner rejects claims 11, 27, 28, 34-38, 40, 43 and 47 under 35 U.S.C. 112, second paragraph, as indefinite.

A. Claim 11

The Examiner has rejected claim 11 as indefinite because of the limitation "the animal." The Examiner suggests that amending the claim such that it is directed to the method of claim 1 wherein the animal cell tissue or organ is a mouse cell tissue or organ would obviate this rejection. Claim 11 has been amended to have the correct antecedent basis. This ground for rejection is therefore moot. Applicants respectfully request that this ground for rejection be withdrawn.

B. Claim 27

The Examiner has rejected independent claim 27, and dependent claims 28, 34-38, 40, 43 and 47, as indefinite as derivatives of the target gene.

The Examiner asserts that the specification defines "derivatives" as any isolated nucleic acid molecule which contains significant sequence similarity to said nucleotide sequence or part thereof. The Examiner alleges that the metes and bounds of the limitation are unclear because the specification provides no objective measure of "significant sequence similarity, and that it is therefore impossible to tell what is encompassed by a derivative of a target gene."

Without acquiescing or admitting to the Examiner's rejection, Applicants have amended the claim 27 to remove reference to "derivatives." This ground for rejection is now moot. Applicants respectfully request that this ground for rejection be withdrawn.

C. Claim 28

The Examiner alleges that claim 28 is indefinite in its recitation of "the animal cell, tissue, organ or organism." This claim limitation now has antecedent basis in claim 27, which recites "an animal cell, tissue, organ or organism." This ground for rejection is therefore moot. Applicants respectfully request that this ground for rejection be withdrawn.

D. Claim 35

The Examiner alleges that claim 35 is indefinite, since it is directed to the synthetic gene according to claim 24, while claim 24 is directed to a method.

Through a clerical error, claim 35 depended through claim 24. Claim 35 has been amended to depend from claim 34. Given that antecedent basis is provided for every limitation of claim 35, it is clear that the amendment merely makes explicit what was inherent in the claim.

This ground for rejection is therefore moot. Applicants respectfully request that this ground for rejection be withdrawn.

Double Patenting

The Examiner provisionally rejects claims 27 and 38 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 2 of copending Application No. 09/100,812. Claims 43 and 47 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 38 of copending Application No. 09/100,812.

Since this is a provisional obviousness-type double patenting rejection, Applicants respectfully request that this ground for rejection be held in abeyance upon notification of allowable subject matter.

Rejection under 35 USC § 102

A. ***Rejection under 35 USC § 102 of Claims 27, 28, 36 and 37***

The Examiner rejects claims 27, 28, 36 and 37 under 35 U.S.C. 102(b) as being anticipated by Dorer *et al.* (1994) 77:993-1002 (made of record in the IDS filed 14 May 2001).

Claim 27 recites "a synthetic gene comprising: a dispersed or foreign deoxyribonucleotide molecule including one or more tandem repeats of a nucleotide sequence; wherein the synthetic gene is capable of post-transcriptionally repressing, reducing, or otherwise the expression of the target gene." (Emphasis added.) Claims 28, 36, and 37 depend from claim 27.

Dorer et al. fail to teach all limitations of the amended claims.

First, Dorer et al. fail to teach that the disclosed sequences are "capable of post-transcriptionally repressing, reducing, or otherwise the expression of the target gene." (Emphasis added.) Dorer et al. disclose an exclusively transcriptional silencing effect, in which tandem inverse copies correlate to formation of heterochromatin. Association with heterochromatin reduces transcription of genes by preventing RNA polymerases from having access to the genes. Dorer et al. "suggest repression occurs because the paired promoters are sequestered from positive regulators, just outside the transposon, that drives low level expression characteristic of single copy lines." Dorer et al., page 999, second column, and Figure 7. Further, Dorer et al. "propose that pairing multiple closely linked copies of P[*lacW*] produces folded structures recognized by heterochromatin-specific proteins." The transcription of genes spatially linked to heterochromatin proteins is thus reduced.

The present application is directed to "post-transcriptionally repressing, delaying or otherwise reducing the expression of a target gene." The method described by Dorer et al. has no effect on post-transcriptional regulation. Transcription occurs in the nucleus at the transcription site, and is repressed by formation of heterochromatin; post-transcriptional regulation is not affected by secondary or tertiary heterochromatin structure of the transcribed DNA. In the teachings of Dorer et al., transcription of the target gene is reduced, and the formation of heterochromatin has no post-transcriptional effect on the mRNA transcript. The effect taught by Dorer et al. does not post-transcriptionally reduce expression of the target gene. Dorer et al. therefore fail to meet every limitation of claim 27.

Second, Dorer et al. describe the effect of repeated copies of a transgene on its own expression. The repeats were generated by multiple insertions of a single copy of the transgene within a transposon. Dorer et al. provides no reference to delaying, repressing or otherwise reducing expression of a target gene by a separate genetic construct.

Since Dorer et al. fail to meet every limitation of claim 27, Dorer et al. also fail to meet every limitation of claims 28, 36 and 37, which depend from claim 27.

This ground for rejection is therefore moot. Applicants respectfully request that it be withdrawn.

B. Rejection under 35 USC § 102 of Claims 1, 2, 12, 17, 18, 27, 28, 38, 43 and 47

The Examiner rejects claims 1, 2, 12, 17, 18, 27, 28, 38, 43 and 47 under 35 U.S.C. 102(e) as being anticipated by U.S. Patent No. 6,506,559 to Fire *et al* (U.S. Patent '559).

Claims 1, 2, 12, 17, 18, 27, 28, 38, 43 and 47

Amended claim 1 is directed to "a method of post-transcriptionally repressing, delaying or otherwise reducing the expression of a target gene in an animal cell, tissue or organ, said method comprising: introducing to said animal cell, tissue or organ one or more dispersed or foreign deoxyribonucleotide molecules." (Emphasis added.) The claim is thus directed to introducing a DNA to the animal cell, tissue, or organism in which the target gene is post-transcriptionally repressed, delayed, or otherwise reduced. Claims 1, 12, and 17 depend from claim 1.

Claim 18 is directed to "a method of post-transcriptionally repressing, delaying or otherwise reducing the expression of a target gene in an animal cell, tissue or organ, ... and expressing said synthetic gene in said animal cell, tissue, or organ." (Emphasis added.)

Amended claim 27 recites "a synthetic gene ... wherein the synthetic gene is capable of post-transcriptionally repressing, delaying, or otherwise reducing expression of said target gene when expressed in an animal, tissue, or organ." (Emphasis added.) Claims 28, 38, 43, and 47 depend from claim 27.

Fire et al. fail to anticipate the amended claims. The claims have been amended to be limited to introducing DNA to the animal cell, tissue, or organ in which expression of the target gene is repressed, delayed, or otherwise reduced. The Examiner's reasons for rejection are thus obviated by the amendments.

U.S. Patent '559 to Fire et al. does not anticipate repressing, delaying, or otherwise reducing the expression of a target gene in an animal cell, tissue, or organ by introducing DNA to an animal cell, tissue, or organ, as required by the amended claims. Instead, '559 only discloses methods of gene silencing by introducing dsRNA directly. At column 5, lines 8-14, for example, Fire et al. disclose that "the RNA may be directly introduced into the cell (i.e.

intracellularly); or introduced extracellularly into a cavity, interstitial space, or circulation of an organism, introduced orally, or may be introduced by bathing an organism in a solution containing RNA." (Emphasis added). Fire et al. teach that the RNA may be expressed by a food source of an organism prior to introduction of the food source to the organism. Furthermore, the sole example provided in the patent discloses gonadal microinjection of dsRNA to nematodes. The disclosure provided by '559 is therefore limited to direct introduction of dsRNA, or introduction of dsRNA via a food source.

Furthermore, Fire et al. do not anticipate the present claims as of its earliest priority date. Fire et al. claim priority to U.S. Provisional Application No. 60/068,562 (U.S. Prov. Appn. No. '562). U.S. Patent No. '559 to Fire et al. anticipates the present patent application under 35 U.S.C. 102(e) only to the extent that U.S. Prov. Appn. No. '562 anticipates each and every limitation of the claims.

U.S. Prov. Appn. No. '562 fails to disclose introducing DNA *in vivo* to the animal cell, tissue, or organ in which the expression of the target gene repressed, delayed, or otherwise reduced. At most, '562 teaches that the dsRNA may be produced prior to introduction of the dsRNA directly to a cell. Specifically, U.S. Prov. Appn. No. '562 states that "RNA may be synthesized either *in vivo* or *in vitro*." (See U.S. Prov. Appn. No. '562, page 7, lines 10-15.) The provisional application limits production of the dsRNA to production of a sense and antisense RNA transcript, which are then annealed to form dsRNA. (See U.S. Prov. Appn. No. '562 page 15, lines 5 and 15-16.) The provisional application separately discusses introducing dsRNA directly, stating that "the RNA may be introduced into the cell (i.e. intracellularly); or introduced extracellularly into a cavity, interstitial space, or circulation of an organism." (U.S. Prov. Appn. No.'562, page 7, lines 16-17.) As in U.S. Patent No. '559, the sole example provided in U.S. Prov. Appn. No. '562 discloses gonadal microinjection of dsRNA to nematodes. (U.S. Prov. Appn. No.'562, page 16, lines 18-19). Fire et al. thus clearly fail to contemplate introducing DNA to the animal cell, tissue, or organ in which expression of a target gene is repressed, delayed, or otherwise reduced.

The Examiner's reasons for rejection are obviated by the amendment. U.S. Patent '559 to Fire et al. fails to teach every limitation of the claims. Moreover, the priority document U.S. Prov. Appn. No. '562 fails to teach every limitation of the rejected claims. U.S. Patent '559 therefore fails to anticipate the rejected claims under 102(e).

Applicants respectfully request that this ground for rejection be withdrawn.

CONCLUSION

Since the grounds for rejection are now moot, Applicants respectfully request a

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, Applicant(s) petition(s) for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no.**546322000321**.

Respectfully submitted,

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